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Early Biochemical Detection of Adverse Effects of a Neurobehavioral Teratogen Influence of Prenatal Methylmercury Exposure on Ornithine Decarboxylase in Brain and Other Tissues of Fetal and Neonatal Rat

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16. ABSTRACT

Ornithine decarboxylase (ODC), an enzymatic regulator of macromolecule synthesis, has proven useful as a biochemical marker for teratologic events. Daily administration of methylmercury (0.5 or 1 mg/kg s.c.) to pregnant rats during the second and third trimesters had a profound effect on ODC in whole fetus which was detectable as early as 13 days of gestation. Levels of enzyme activity in fetal brain also showed a marked increase centered about gestational day 17, as well as a significant elevation during early postnatal life; in the latter case, the cerebellum appeared to be a major target for methylmercury-induced aberrations. These effects were accompanied by little or no alterations in general growth rate, brain weights, or weights of other tissues (liver, heart, lung). Furthermore, no other tissue displayed such dramatic effects on ODC activity. Lowering the dose of methylmercury by an order of magnitude (0.05 to 0.1 mg/kg), levels which are associated with almost purely neurobehavioral effects of the teratogen, still resulted in clear-cut elevations of both whole fetus ODC and fetal and neonatal brain ODC. These results indicate that a sensitive biochemical detection procedure used in the fetus/neonate can successfully predict the subsequent tissue-specific neurobehavioral damage resulting from methylmercury.

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Early Biochemical Detection of Adverse Effects of a Neurobehavioral Teratogen: Influence of Prenatal Methylmercury Exposure on Ornithine Decarboxylase in Brain and Other Tissues of Fetal and Neonatal Rat

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ABSTRACT Ornithine decarboxylase (ODC), an enzymatic regulator of macromolecule synthesis, has proven useful as a biochemical marker for teratologic events. Daily administration of methylmercury (0.5 or 1 mg/kg s.c.) to pregnant rats during the second and third trimesters had a profound effect on ODC in whole fetus that was detectable as early as 13 days of gestation. Levels of enzyme activity in fetal brain also showed a marked increase centered about gestational day 17 as well as a significant elevation during early postnatal life; in the latter case, the cerebellum appeared to be a major target for methylmercury-induced aberrations. These effects were accompanied by little or no alteration in general growth rate, brain weights, or weights of other tissues (liver, heart, lung). Furthermore, no other tissue displayed such dramatic effects on ODC activity. Lowering the dose of methylmercury by an order of magnitude (0.05 to 0.1 mg/kg), levels which are associated with almost purely neurobehavioral effects of the teratogen, still resulted in clear-cut elevations of both whole fetus ODC and fetal and neonatal brain ODC. These results indicate that a sensitive biochemical detection procedure used in the fetus/neonate can successfully predict the subsequent tissue-specific damage to neurotransmitter systems and behavior resulting from methylmercury.

Although acute intoxication with organomercurials such as methylmercury results in widespread damage to a variety of organ systems, chronic exposure to lower levels produces symptoms associated primarily with neurological damage (reviews, Reuhl and Chang '79; Chang and Annau, '84). The sensitivity of the nervous system to methylmercury, combined with the vulnerability of the developing brain to perinatal insult, has led to the definitive identification of this compound as a neurobehavioral teratogen. During ontogenetic development, both central and peripheral neuronal systems appear to be affected (Taylor and DiStefano, '76; Bartolome et al '82a, '84a,b), with gross alterations in brain structure and function (in addition to generalized morphological abnormalities) at clearly toxic doses (Chang et al., '77; Choi et al., '80; Reuhl and Chang, '79; Chang and Annau, '84). Even when the dose of methylmercury is below the threshold for classical teratologic alterations, more subtle behavioral teratogenic effects are still detectable; indeed, repeated administration of as little as 0.5 mg/kg or single treatments of up to 10 mg/kg produce clear-cut abnormalities in open-field and swimming behaviors as well as in learning (Reuhl and Chang, '79; Chang and Annau, '84).

The identification of biochemical mechanisms that underlie the adverse behavioral effects of early methylmercury exposure has proven elusive; organomercurials cause generalized disturbances in cell function that can arise from alterations in polyamine, protein, and nucleic acid synthesis; phospholipid metabolism; mitochondrial structure; and respiration and membrane permeability (Vallee and Ullmer, '72; Clarkson, '72; Bru-

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baker et al., '73; Chang et al., '74; Fowler and Woods, '77; Menon et al., '78; Mehra and Kanwar, '80; Bartolome et al., '82b). These types of effects are common to a variety of tissues rather than displaying specificity for neuronal systems; however, the threshold for many of the biochemical anomalies lies within range of that for neurobehavioral (0.1 to 10 mg/kg in repeated or single doses; Reuhl and Chang, '79) damage. The question remains, then, as to whether a biochemical teratologic event can be identified that meets the following criteria: (1) it involves neural tissues more than others; (2) it represents a metabolic process which participates definitively in maturation of brain tissue; (3) it is detectable before the onset of neurobehavioral alterations; and (4) it occurs at the low dose levels that typify behavioral teratogenesis. In the current study, we have explored the possibility that ornithine decarboxylase (ODC) might fulfill these requirements. ODC catalyzes the initial step in synthesis of polyamines, intracellular messengers that regulate macromolecule synthesis during replication, differentiation, and growth (reviews, Bachrach, '73; Russell and Durie, '78; Slotkin, '79; Russell, '80; Slotkin and Thadani, '80). Each tissue has its own archetypical ODC developmental pattern, and numerous studies have shown that drugs, hormones, or environmental conditions that perturb the ODC/polyamine system during critical maturational periods invariably produce defective cellular development and eventual functional disturbances (Slotkin and Thadani, '80; Bartolome et al., '82b; Slotkin et al., '82, '83, '84). Consequently, we have examined the sensitivity and selectivity of effects of prenatal methylmercury exposure on ODC in the developing fetus and neonate.

METHODS

Animal treatments

Timed pregnant Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA) were shipped by climate-controlled truck at 3–4 days of gestation; transit time was approximately 12 hr. After arrival, animals were housed individually in breeding cages and allowed food and water ad libitum. Beginning at 8 days of gestation, dams were given methylmercury hydroxide (0.05, 0.1, 0.2, 0.5, or 1 mg/kg s.c., obtained from Alfa Products [Danvers, MA] as a 1 M solution in water, purity > 97%) daily, continued until the day before birth (birth was considered to occur on gestational day 22); control animals received daily injections of water equal in volume to the methylmercury injections (1 ml/kg adjusted to pH 6.0–6.5, the same as the methylmercury solutions). Each treatment group contained at least 20 pregnant dams. Immediately after birth, pups from all litters were randomized within their respective treatment groups and redistributed to the nursing mothers, with culling by random selection to litter sizes of 10 or 11 pups (in order to maintain a standard nutritive status). Randomization was again carried out at intervals of several days, and in addition, pups of both sexes were chosen for each experiment from several different cages for each group. Animals were weighed and killed by decapitation at 3, 6, 8, 10, and 17 days postnatally and tissues weighed prior to analysis. For studies in fetuses, dams were selected and decapitated at 13, 15, 17, and 20 days of gestation, and fetuses were removed and dissected; the time from abdominal incision to completion of tissue weighing and homogenization took less than 3 minutes for each dam and in no case was a difference seen in activity between the first fetus and last fetus.

Ornithine decarboxylase activity

Tissues were homogenized in 19–39 volumes of 10 mM Tris (pH 7.2) and ODC activity was determined in duplicate in the 26,000g supernatant by a modification (Lau and Slotkin, '80) of the method of Russell and Snyder ('68). The incubation medium contained final concentrations of 50 μM pyridoxal-5′phosphate, 1.5 mM dithiothreitol, and 4.5 μM l-[1-^{14}C]ornithine (53 mCi/mmol, New England Nuclear, Boston MA).

Statistics

Developmental profiles for each of the variables measured were compared in methylmercury-exposed and control rats by two-way analysis of variance (factor 1 = age, factor 2 = treatment). A criterion of $p < 0.05$ was considered to indicate statistical significance. Evaluations were made both upon the individual animals and by litters; results were equivalent either way. In light of the fact that no litter-to-litter or sex differences were found within treatment groups, and in view of the randomization procedures described above, results are presented based on the individual values.

RESULTS

Administration of 0.5 or 1 mg/kg of methylmercury daily to pregnant rats had very

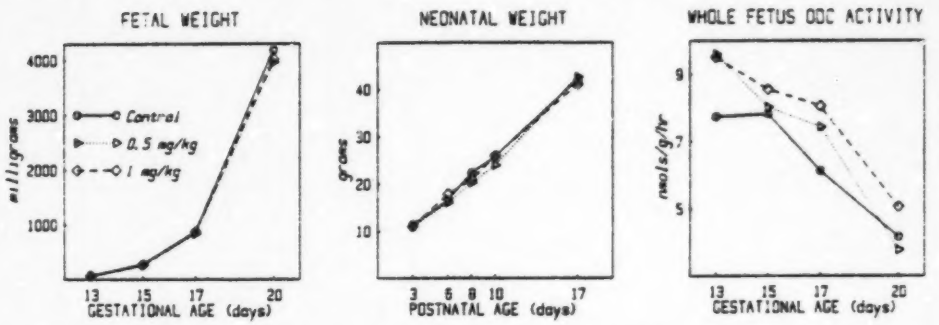

Fig. 1. Effects of maternal methylmercury administration on development of fetal and neonatal body weights and whole fetus ODC activity. Data represent means of values from 8 to 22 fetuses obtained from 4 to 8 dams at each age for each treatment. Standard errors were < 3% for weights and < 5% for ODC. Two-way ANOVA indicates a significant effect of methylmercury on both weight and ODC, as well as significant interactions of age × treatment.

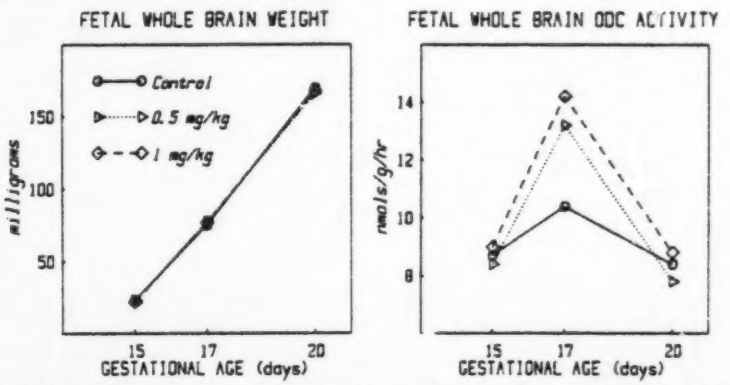

Fig. 2. Effects of maternal methylmercury administration on fetal brain weights and whole brain ODC activities. Data represent means of values from 6 to 10 fetuses obtained from 4 to 8 dams at each age for each treatment. Standard errors were < 3% for weights and < 5% for ODC. Two-way ANOVA indicates a significant effect of methylmercury on brain ODC activity and a significant interaction of age × treatment; differences in brain weights were not significant.

little effect on body weights of either fetuses or neonates (Fig. 1); differences, although statistically significant, never amounted to more than 5%. In contrast, there were dramatic effects of methylmercury exposure on fetal ODC activity, with marked elevations apparent throughout gestational days 13 to 17. A more selective effect was apparent for fetal brain (Fig. 2), where tissue ODC activity was elevated specifically at gestational day 17. Individual brain regions were examined in the perinatal period to determine if effects persisted beyond the fetal stage and if they were confined to a specific locus (Fig. 3). The cerebellum appeared to be the most sensitive region, with clear-cut increases in activity apparent at both doses in both the immediate prenatal period and through the first two weeks of postnatal life. Midbrain + brainstem and cerebral cortex displayed much less sensitivity, with no definitive changes in the immediate perinatal period and increases of small magnitude in the first postnatal week. In only one case (midbrain + brainstem) was there any effect on regional brain weights, and even in that case the effect of methylmercury was to cause a small *increase*, not decrease, in the high dose group.

Increases in ODC activity were still discernible in the fetus even when the dosage was reduced to 0.05 or 0.1 mg/kg (Table 1).

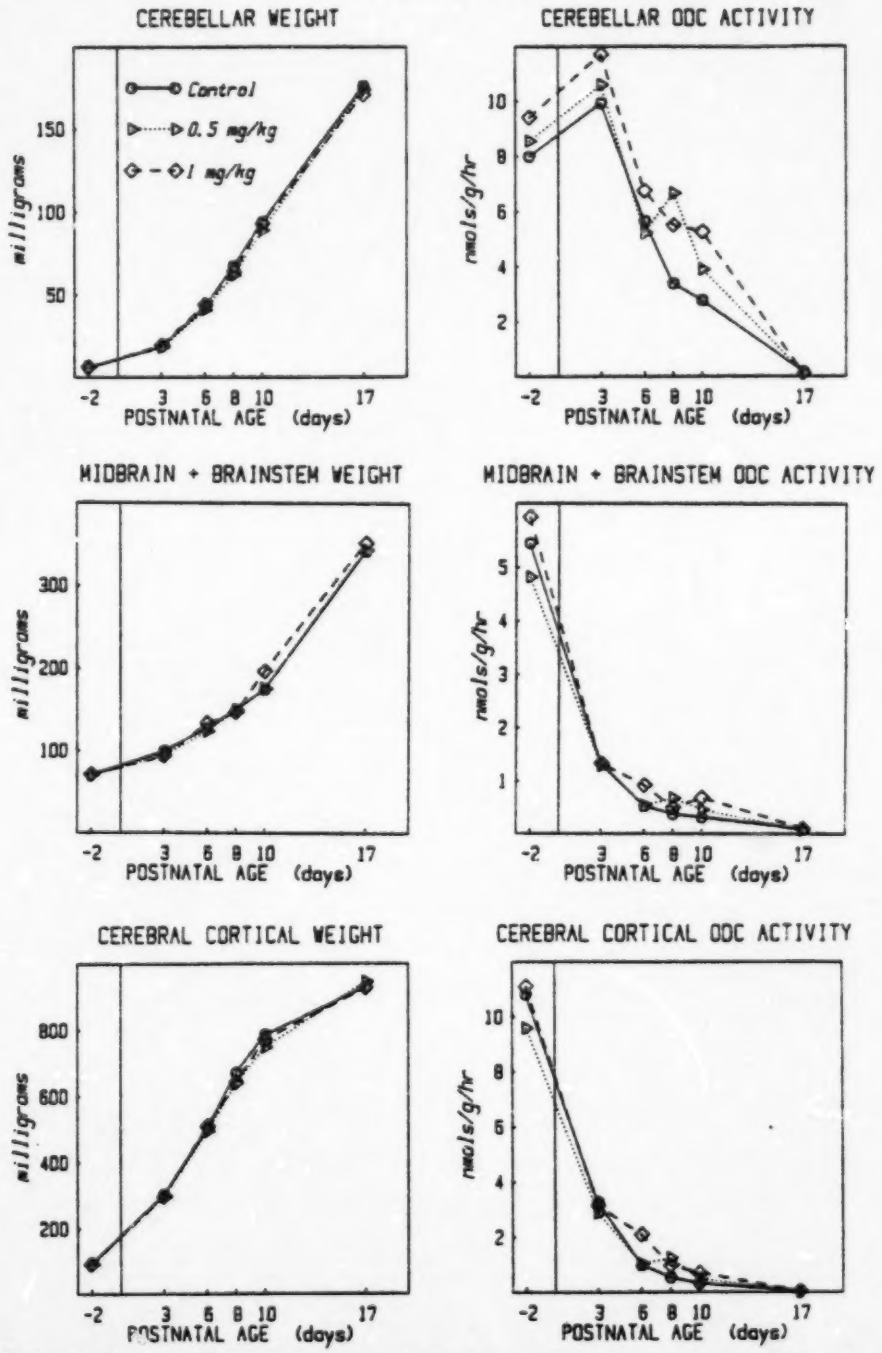

Fig. 3. Effects of maternal methylmercury administration on development of brain region weights and ODC activities. Data represent means of values from 5 to 16 fetuses (4 dams per treatment group) or neonates at each age for each treatment. Two-way ANOVA indicates significant effects of methylmercury on ODC activity in all three regions with a significant age × treatment interaction for midbrain + brainstem and cerebral cortex. Significant effects on tissue weight were seen only in midbrain + brainstem.

TABLE 1. Effects of low doses of methylmercury on fetal and brain ODC[1]

Treatment: Whole fetus	ODC activity (nmoles/gm/hr) 13 days gestation	17 days gestation
Control	7.74 ± 0.36	6.13 ± 0.30
0.05 mg/kg	8.20 ± 0.50	8.51 ± 0.39
0.1 mg/kg	8.51 ± 0.62	6.98 ± 0.30
Whole brain	**17 days gestation**	**6 days postnatal**
Control	10.4 ± 0.4	1.28 ± 0.06
0.05 mg/kg	14.6 ± 1.0	1.82 ± 0.08
0.1 mg/kg	12.0 ± 0.8	1.72 ± 0.18

[1]Data represent means and standard errors of determinations from 10 to 22 fetuses obtained from 4 to 8 dams in each treatment group at each age (top), or 10 fetuses from 4 dams per treatment group (bottom). Two-way ANOVA indicates significant overall effects of methylmercury ($p < 0.01$) on both variables.

Equally important, both the gestational and early postnatal increases in brain ODC were clearly demonstrable even at the lowest dose employed; indeed, the effect appeared to be maximal even at 0.05 mg/kg, which produced the same magnitude of ODC increase as the highest dose employed (1 mg/kg, Fig. 2).

The results obtained in fetal brain and neonatal cerebellum can be contrasted with the essentially negative findings in other, nonneural tissues (Fig. 4). Liver ODC exhibited only small increments of activity at 1 mg/kg and no change at the lower dose. Heart ODC was essentially normal except at 3 days of postnatal age in the high dose group, and lung ODC activity was unchanged or even slightly reduced. Again, there was little or no effect on tissue weights with either dose of methylmercury.

The methylmercury regimens employed here did not cause any overt signs of maternal toxicity and had no significant effects on litter size or viability.

DISCUSSION

The susceptibility of the fetus to neurological damage by methylmercury has led to the general acceptance of this compound as a behavioral teratogen. The basis for behavioral alterations in offspring of methylmercury-treated mothers can be readily visualized when high doses of the compound are used (Chang et al., '77; Reuhl and Chang, '79; Chang and Annau, '84); under these circumstances, structural damage to brain tissues is apparent and can account for functional impairment. Several investigations have indicated that behavioral anomalies can be detected even in the immediate postnatal period at doses of methylmercury that compromise morphological development (> 1 mg/kg); of equal importance is the observation that even low doses of the compound can still produce subtle changes in performance in later life (Spyker et al., '72; Hughes and Annau, '76; Hughes and Sparber, '78; Reuhl and Chang, '79; Eccles and Annau, '82a,b; Chang and Annau, '84). These reports raise the question of identification of the potential mechanisms by which such delayed events come about. The current study lends support to the view that behaviorally teratogenic doses of methylmercury produce early abnormalities of brain cell development as exemplified by the effects on ODC. Thus, marked elevations of brain ODC were detectable in the fetus during the last trimester, a developmental period in which major stages of brain growth are occurring and, therefore, one in which there is potential behavioral impact. Abnormal ODC levels could also be detected in specific brain regions in the immediate postnatal period and, indeed, the region most affected (cerebellum) is also one that develops relatively late and is a highly sensitive target for methylmercury (Reuhl and Chang, '79; Chang and Annau, '84). Higher doses of the compound are known to produce specific structural damage in cerebellum (Chang et al., '77; Reuhl et al., '81), a finding in keeping with our observation that this region is quite sensitive to lower doses of methylmercury. It should be noted that some of the effect on ODC after birth could reflect actions of residual methylmercury, since elevations of brain ODC are found after postnatal treatment (Bartolome et al., '82b).

In view of the clearly-defined role of the ODC pathway in cellular maturation, the methylmercury-induced alterations in enzyme activity are likely to participate in the subsequent abnormalities of function. ODC and the polyamines regulate nucleic acid and

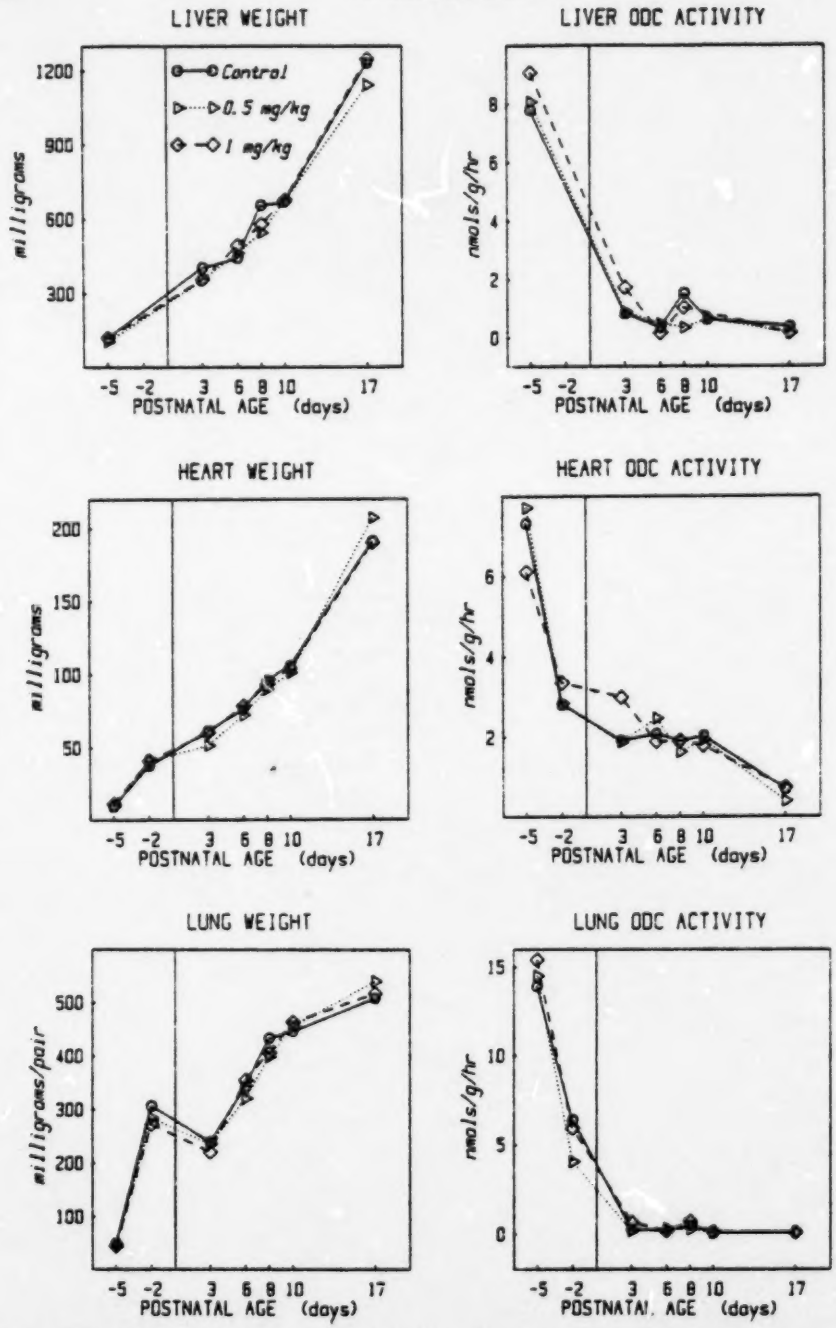

Fig. 4. Effects of maternal methylmercury administration on growth and ODC activity in non-neural tissues. Data represent means of values from 5 to 16 fetuses (4 to 8 dams per treatment group per age point) or neonates at each age for each treatment. Two-way ANOVA indicates significant effects of methylmercury on liver weight and ODC and on lung ODC; significant age × treatment interactions were seen for liver ODC, heart ODC, and lung ODC.

protein synthesis during replication and differentiation (reviews, Bachrach, '73; Russell and Durie, '78; Slotkin, '79; Russell, '80; Slotkin et al., '84); thus, previous observations of abnormalities in macromolecular synthesis of methylmercury-exposed animals (Joiner and Hupp, '79; Choi et al., '80) may reflect direct biologic end-points of ODC alterations. Indeed, methylmercury has been found to produce a spectrum of long-term neurochemical effects that display regional selectivities similar to those of the early actions on ODC (Bartolome et al., '82a,b, '84a,b, '85; Slotkin et al., '85a,b).

Methylmercury concentrates in a number of tissues in addition to the nervous system (Swensson and Ulfvarson, '68; Omata et al., '78; Chen et al., '79). High doses of the compound produce damage to liver, kidney, heart, and other organs and it is thus important to determine whether the alterations in ODC caused by low doses of methylmercury are restricted to the brain. Examination of ODC patterns in liver, heart, and lung in the period immediately preceding and following birth failed to reveal major changes, thus confirming the selectivity of effect. Be that as it may, whole fetus ODC values were clearly abnormal at developmental stages (gestational days 13, 15) preceding the change in brain ODC; in fact, the same range of low doses of methylmercury that produce primarily neurobehavioral alterations (Reuhl and Chang, '79; Chang and Annau, '84) and brain ODC alterations also caused comparable earlier perturbations of fetal ODC. Thus, the eventual impact of methylmercury on central nervous system development during late gestation and postnatal life may result from alterations in metabolic events much earlier in prenatal development.

In conclusion, these studies show that ODC provides an early and reliable biochemical marker for establishing the presence of brain damage from methylmercury. Abnormalities of ODC in whole fetus, brain, and specific brain regions can be identified even at doses low enough to be regarded as nearly exclusively neurobehaviorally teratogenic. The finding that biochemical perturbations can exhibit exquisite sensitivity to subtle teratogenic insult indicates the potential value of such approaches as an adjunct to more classical morphological and behavioral teratogenic techniques.

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